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The Mating Ability of Males of *Culex pipiens fatigans* Wiedemann Sterilized with Apholate or Tepa

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The chemosterilants apholate and tepa are known to induce a high degree of sterility in the males of Culex pipiens fatigans. *The studies reported show that 36-hour-old apholate- and tepa-sterilized laboratory-bred males can inseminate the same number of laboratory-bred or wild females as can normal laboratory or wild males in the first 48 hours of their lives. Males sterilized by either compound were found to be more competitive in mating with normal laboratory females than were the normal laboratory males. When normal virgin females were mated first with sterile and then with normal males or* vice versa, *the sperms of the first mating always decided the fate of eggs. Apholate-sterilized laboratory males were not only compatible with wild females but could even induce the same level of sterility in them and were fully as competetive in mating as normal wild males. The authors stress that it has still to be ascertained whether these encouraging laboratory findings would apply in field conditions.*

A large number of chemosterilants have been shown in the course of laboratory research to be highly effective in inducing sterility in the tropical house mosquito, *Culex pipiens fatigans* Wiedemann by various methods of application (Mulla, 1964; Das, 1967; Grover, Pillai & Dass, 1967; McCray & Schoof, 1967; Pillai & Grover, 1969). However, for successful application of these chemosterilants in the field using the sterile-male technique, males sterilized by these compounds must retain their sexual vigour to compete with normal wild males for normal virgin wild females, and in polygamous females, sperms from sterilized males must compete with those of normal males.

Numerous reports are now available of instances in which chemosterilants did not adversely effect the mating ability of houseflies (*Musca domestica* L.), moquitos (*Aedes aegypti* (L); *Anopheles quadrimaculatus* Say.; *C. p. fatigans*), screw-worm flies (*Cochliomyia hominivorax* (Coquerel)), pink boll worm (*Pectinophora gossypiella* (Saunders)), green sheep blowfly (*Phaenicia sericata* (Meigen)), and several other insects (see LaBrecque, Meifert & Smith, 1962; LaBrecque et al., 1966; Ascher & Avdat, 1967; Schmidt, Dame & Weidhaas, 1964; Weidhaas & Schmidt, 1963; White, 1966; Grover & Pillai, 1970; Murray & Bickley, 1964; Das, 1967; Smittle et al., 1968; Crystal, 1965, 1969; Ouye et al., 1965; Millar, 1965). On the contrary, some of these chemosterilized males were reported to be more strongly competitive than normal males in mating with normal females (Ascher & Avdat, 1967; LaBrecque, Meifert & Smith, 1962; Millar, 1965; Crystal, 1965, 1969; White, 1966; Grover & Pillai, 1970; Das, 1967). On the other hand, reports are also available indicating that chemosterilization has resulted in loss of vigour in mating or in mating competitiveness of male mosquitos (*Ae. aegypti*, *An. quadrimaculatus*), boll weevil (*Anthonomous grandis* Boheman), tsetse fly (*Glossina morsitans* Westwood), housefly and screw-worm fly (Dame, Woodward & Ford, 1964; Dame & Schmidt, 1964; Schmidt, Dame & Weidhaas, 1964; Davich et al., 1965; Simpson, 1958; Chang, 1965; Crystal, 1964a, 1964b).

Pillai & Grover (1969) report that non-toxic doses of 10 ppm apholate applied at the larval stage and 3580 ppm tepa applied at the pupal stage induced

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82% and 97% sterility, respectively, in males of *C. p. fatigans*. Some data are available on the effect of apholate on the mating competitiveness of *C. p. fatigans* males treated at the larval stage (Murray & Bickley, 1964). However, detailed laboratory evaluation of the mating ability of males of this mosquito sterilized by apholate or tepa is wanting. Moreover, the results of a number of workers using chemosterilants or radiation in field studies of mosquitos have not been very encouraging owing to the effect of certain factors such as behavioural differences and differences in mating ability between the laboratory and the wild strain (Weidhaas, Schmidt & Seabrook, 1962; Morlan, McCray & Kilpatrick, 1962; Dame, Woodward, Ford & Weidhaas, 1964). The present studies were therefore directed to obtaining the laboratory data on the mating ability of *C. p. fatigans* chemosterilized by apholate or tepa, that are basic to any field application.

MATERIALS AND METHODS

The strain of *C. p. fatigans* used in the present investigation originated from mosquitos collected from Delhi in October 1965 and since then colonized in our laboratory as reported earlier (Pillai & Grover, 1969; Grover & Pillai, 1969). For wild populations of this mosquito, fresh collections of 4th-instar larvae or pupae were made locally during September–October 1967. Such larvae were reared until pupation in the same waters as those in which they had been found breeding. Newly emerged pupae were collected, sexed and then isolated singly in small glass vials for emergence. The adults that emerged from these pupae were used for mating-competitiveness tests after proper identification.

Laboratory populations were sterilized by exposing 2nd-instar larvae to 10 ppm apholate until pupation or by exposing newly emerged pupae to 3580 ppm tepa for 28 hours as described in an earlier paper (Pillai & Grover, 1969). After the treatment period, pupae were collected, rinsed in distilled water, sexed and then transferred to small emergence vials. All experiments were conducted in screened mosquito cages measuring 18 in × 16 in × 16 in (45 cm × 40 cm × 40 cm) and provided with cloth sleeves. During the experimental period adults were fed on 1% glucose solution in soaked cotton pads which were renewed every other day. Unless otherwise stated, the females used in all experiments were 2–3 days old and the males 2 days old. Females were provided with a pigeon-blood meal after being allowed sufficient time for mating. Females in each cage were allowed to lay egg-rafts in a small enamel bowl containing distilled water. The egg-rafts laid each day were collected, recorded and hatched individually in separate glass vials. Eggs hatched within 48 hours were used for calculating the percentage sterility.

Detailed procedures for the different experiments conducted are as follows.

Experiment 1. Insemination frequency of normal laboratory females by laboratory males sterilized by apholate or tepa in comparison with that by normal males

For this study 50 sterilized males, 36 hours old, were caged with 300 virgin females (ratio 1 : 6) and allowed to mate for a period of 48 hours. The males were then removed and 100 females were taken at random from the cage, dissected in *Ae. aegypti* saline (Hayes, 1953) and examined for sperms in the spermathecae. An identical control using normal laboratory males and normal females was run simultaneously. The total number of females inseminated in each cage was recorded and the number of females inseminated per male in 48 hours was calculated. Each experiment was replicated four times.

Experiment 2. Mating competitiveness of laboratory males sterilized by apholate or tepa in competition with normal laboratory males for virgin laboratory females

Sterilized males combined in various ratios (shown in Tables 2 and 3) with normal laboratory males were allowed to compete for mating with 50 virgin laboratory females. Both the treated and the normal males were released into the cages one day earlier than the females, so that both types could position themselves at random inside the cages. All females in each cage were released together at the same time. The releases were done a little before dusk, so that the mosquitos might start mating soon. Three days were allowed for mating;[1] after that time the females were given a blood meal twice weekly. The egg-rafts laid each day were collected and allowed to hatch individually in glass vials. Egg-rafts laid during the first 15 days from the start of the experiment were used to assess the percentage hatch. Twenty egg-rafts taken at random from those laid as a result of each cross were used for analysis of indi-

[1] Males were not removed after the three days.

vidual egg-raft hatches. Each experiment was replicated twice. Treated males were dusted with aluminium powder for easy identification of those that died. The mortality among the treated and control males was found to be less than 3%. Dead males were replaced by similar treated or untreated males from reserves that had been maintained in an identical manner.

Experiment 3. Effect of mating sequence on the egg-hatch of normal females mated with apholate-sterilized and normal males

One hundred virgin females were placed in each of four cages and 400 treated or control males were released into each cage. Females were allowed to mate with these males for 48 hours and the males were then replaced by a fresh batch of 400 treated or normal males for a second 48-hour mating period (shown in Table 4). At the end of the second mating period, the males were removed from all the cages, and the females given a blood meal on the same day and another the next day. After the blood meal, females were allowed to lay eggs. The number of egg-rafts laid in each cage was recorded and 20 egg-rafts collected at random were allowed to hatch individually. The numbers of eggs in each of these rafts and the numbers of larvae hatched were also recorded and the percentage hatch for each egg-raft was calculated. These rafts were grouped in different hatch ranges for analysis.

Experiment 4. Insemination frequency of wild females by laboratory males sterilized by apholate or tepa in comparison with that by wild males

Fifty 36-hour-old normal laboratory males or sterilized laboratory males were allowed to mate with 300 wild females (ratio 1 : 6) for 48 hours. The procedure was similar to that in experiment 1 except that in the control cross wild males were crossed with wild females. The number of females inseminated per male in 48 hours was calculated.

Experiment 5. Mating competitiveness of apholate-sterilized laboratory males in competition with normal wild males for normal wild females

As a first step, 50 wild males were crossed with 50 wild females to assess the oviposition rate and the percentage egg hatchability of the wild population in laboratory conditions. Another cross was made between 50 normal laboratory males and the same number of wild females to see whether laboratory males could fertilize wild females and, if so, to what extent. After this, sterilized laboratory males were mixed with normal wild males in different ratios (shown in Table 6) and allowed to mate with 50 virgin normal wild females. The rest of the procedure was the same as that for experiment 2.

RESULTS

It is apparent from the insemination-frequency results given in Table 1 that laboratory males sterilized by apholate or tepa could inseminate essentially the same number of normal laboratory females in 48 hours as could the normal laboratory males (experiment 1).

TABLE 1
INSEMINATION FREQUENCY BY NORMAL AND STERILIZED LABORATORY MALES OF *C.P. FATIGANS* IN 48 HOURS

Males	No. of females inseminated per male [a]
Apholate-sterilized	3.12 ± 0.276
Tepa-sterilized	3.24 ± 0.349
Normal laboratory	3.06 ± 0.312

[a] Mean values ± standard error.

The results of experiment 2 on the mating competitiveness of apholate- and tepa-sterilized laboratory males in competition with normal laboratory males for normal laboratory females are summarized in Tables 2 and 3. In all crosses, involving different ratios of treated and normal males, the actual percentage sterility induced is slightly higher than the expected value. This clearly shows that laboratory males sterilized by apholate and tepa are more competitive in mating with normal females than are the normal males. The oviposition rate was very similar for all the crosses; the total number of egg-rafts laid varied from 80 to 87 for the experiment with apholate-sterilized males and from 70 to 75 for that with tepa-sterilized males, and the number of eggs laid per female ranged from 224 to 247 and 238 to 248 respectively. Some 14%–15% of egg-rafts resulting from crossing normal males with normal females were in the hatch-range 81%–100%, while 90% or more of egg-rafts resulting from crossing treated males with normal laboratory females were in the hatch-range 0–40%. In different

TABLE 2

MATING COMPETITIVENESS OF APHOLATE-STERILIZED LABORATORY MALES WITH NORMAL LABORATORY FEMALES IN COMPETITION WITH NORMAL LABORATORY MALES FOR A PERIOD OF 15 DAYS

Sex ratio in each series [a] T♂ : N♂ : N♀	Total rafts laid	Average no. of eggs laid/♀	Percentage sterility		Percentage of egg-rafts in indicated hatch-ranges				
			Actual	Expected [b]	0–20 % hatch	21 %–40 % hatch	41 %–60 % hatch	61 %–80 % hatch	81 %–100 hatch
0 : 1 : 1	80	247.4	10	—	—	—	—	15	85
1 : 0 : 1	80	243.4	83	—	75	15	10	—	—
1 : 1 : 1	80	224.1	54	46	37	19	6	7	31
2 : 1 : 1	81	242.3	66	59	43	25	6	6	19
5 : 1 : 1	87	231.5	80	71	53	29	6	—	12
1 : 5 : 1	82	240.2	25	22	15	5	—	10	70
10 : 1 : 1	85	246.1	83	76	75	10	15	—	—

[a] 50 adults of each sex or multiples. T = treated; N = normal.
[b] Corrected for normal sterility in untreated populations of both sexes and for incomplete sterility in treated male populations.

TABLE 3

MATING COMPETITIVENESS OF TEPA-STERILIZED LABORATORY MALES WITH NORMAL LABORATORY FEMALES IN COMPETITION WITH NORMAL LABORATORY MALES FOR A PERIOD OF 15 DAYS

Sex ratios of experiments [a] T♂ : N♂ : N♀	Total rafts laid	Average no. of eggs laid/♀	Percentage sterility		Percentage of egg-rafts in indicated hatch-ranges				
			Actual	Expected [b]	0–20 % hatch	21 %–40 % hatch	41 %–60 % hatch	61 %–80 % hatch	81 %–100 hatch
0 : 1 : 1	70	247.1	9	—	—	—	—	14	86
1 : 0 : 1	71	244.1	93	—	100	—	—	—	—
1 : 1 : 1	75	246.2	59	51	60	—	—	7	33
2 : 1 : 1	72	241.9	71	65	71	—	—	7	22
5 : 1 : 1	73	238.5	85	79	86	—	—	—	14
1 : 5 : 1	72	248.2	27	23	17	—	—	14	69
10 : 1 : 1	74	242.2	90	85	93	—	—	—	7

[a] 50 adults of each sex or multiples. T = treated; N = normal.
[b] Corrected for normal sterility in untreated populations of both sexes and for incomplete sterility in treated male populations.

competitive crosses, egg-rafts with intermediate hatch ranges were not as numerous as had been expected; this suggests that eggs are largely fertilized either by treated or by normal sperms and not by both. In general, an increase in the proportion of sterile males increased the percentage sterility in females, and, similarly, a decrease in the proportion of sterile males decreased the percentage sterility in females.

The effect of mating sequence on the egg-hatch of normal females mated with apholate-sterilized and normal males (experiment 3) is shown in Table 4. The percentages of females that oviposited and the numbers of eggs laid per female were almost similar for all four crosses. Females crossed twice with treated males laid eggs with a hatch of 17% only, while females mated twice with normal males laid eggs having a hatch of 92%. Females first exposed to untreated males and then to sterilized males laid eggs with an 87% hatch. On the other hand, females with the converse exposure sequence laid eggs only 23% of which hatched. The percentage

TABLE 4

EFFECT OF MATING SEQUENCE ON THE EGG HATCH OF FEMALE *C.P. FATIGANS* MATED WITH APHOLATE-STERILIZED AND NORMAL MALES

Type of mating [a]		Females that oviposited (%)	No. of rafts analysed	Average no. of eggs laid/♀	Hatch (%)	Percentage of egg rafts in indicated hatch-ranges				
First mating	Second mating					0–20 % hatch	21 %–40 % hatch	41 %–60 % hatch	61 %–80 % hatch	81 %–100 % hatch
N♂	N♂	88	20	179.2	91.99	—	—	—	15	85
T♂	T♂	90	20	171.9	17.21	75	15	10	—	—
N♂	T♂	89	20	178.3	86.57	—	5	—	15	80
T♂	N♂	87	20	167.9	23.22	65	20	10	—	5

[a] 100 virgin females (2-days old) mated with 400 males in the first mating for 48 hours, the latter being replaced by a further 400 males in the second mating for the next 48 hours. N = normal (2-day-old virgin males); T = treated (2 day-old virgin males).

hatches from third and fourth crosses almost resembled those from first and second crosses, suggesting that most females are fertilized by sperms from the first mating. However, in the last two crosses about 5% of the rafts differed from the majority and it seems that the females that laid these 5% were fertilized by the sperms from the second mating. In this experiment, too, very few egg-rafts with an intermediate hatch-range were laid in the last two crosses; this confirms that in *C. p. fatigans* most of the eggs are fertilized by the first mating.

From Table 5, which gives the insemination frequency of wild females by sterilized males and by normal wild males (experiment 4), it is apparent that the numbers of females inseminated were essentially the same whether they mated with sterilized or with wild males. This shows that males sterilized by either apholate or tepa compare well with wild males for inseminating wild females in 48 hours.

The results of studies of the mating competitiveness of apholate-sterilized males in competition with normal wild males for normal wild females are presented in Table 6. It was observed that wild males could mate with, as well as fertilize, wild females in cages under laboratory conditions, and

TABLE 5

INSEMINATION FREQUENCY BY NORMAL AND STERILIZED LABORATORY MALES AND BY WILD MALES FOR A PERIOD OF 48 HOURS, WHEN CROSSED WITH WILD FEMALES

Males	No. of females inseminated per male [a]
Wild	3.18 ± 0.246
Laboratory	3.00 ± 0.327
Apholate-sterilized	3.24 ± 0.223
Tepa-sterilized	3.30 ± 0.314

[a] Mean values ± standard error.

TABLE 6

MATING COMPETITIVENESS OF APHOLATE-STERILIZED LABORATORY MALES WITH WILD NORMAL FEMALES IN COMPETITION WITH WILD NORMAL MALES FOR A PERIOD OF 15 DAYS

Sex ratio in each series [a]	Total rafts laid	Avearge no. of eggs laid/♀	Percentage sterility	
			Actual	Expected [b]
W♂ : N♂ : W♀				
0 : 1 : 1	73	221.3	14	—
1 : 0 : 1	70	212.0	15	—
T♂ : W♂ : W♀				
1 : 0 : 1	75	190.8	85	—
1 : 1 : 1	76	204.0	53	50
2 : 1 : 1	75	211.0	68	62
5 : 1 : 1	74	201.0	79	73
1 : 5 : 1	72	200.1	28	27

[a] 50 adults of each sex or multiples. W = wild; N = normal; T = treated.

[b] Corrected for normal sterility in untreated populations of both sexes and for incomplete sterility in treated male populations.

that normal laboratory males could mate with and fertilize wild females to the same extent as wild males; this is apparent from the percentage hatch of eggs from these two crosses. In addition, it is interesting to note that sterilized laboratory males could induce the same level of sterility in wild females as in normal laboratory females. The actual percentage sterility induced by sterile males in all the series of mating experiments (i.e., for all the different ratios) was higher than expected. An increase in the proportion of treated males to normal wild males increased the sterility level in the wild population whereas a decrease in the proportion of sterilized to normal wild males resulted in a decrease of sterility in wild females. The oviposition rates in the different crosses were closely similar. The results clearly show that apholate-treated males could fully compete with wild males for mating with wild females.

DISCUSSION

The present studies clearly indicate that the sterilizing doses of the chemosterilants used here did not affect the mating vigour of *C. p. fatigans* as treated laboratory males could compete with normal males in inseminating normal females. The actual sterility induced in all the crosses was found to be higher than theoretically expected. Murray & Bickley (1964) with *C. p. quinquefasciatus* Say (=*C. p. fatigans* Wiedemann) obtained identical results by exposing 4th-instar larvae to 10 ppm or 15 ppm apholate, though the actual sterility in one cross (4 treated males: 1 normal male: 1 normal female) was lower than expected. Also Das (1967) and Smittle et al. (1968) found that males of this mosquito exposed at the adult stage by dusting with apholate were more competitive for normal females than were the normal males. Adult males of *Ae. aegypti* fed an apholate-treated diet were fully as competitive in mating as normal ones (Weidhaas & Schmidt, 1963). Further, the pupal treatment of this species with thiotepa did not affect the mating competitiveness of sterilized males 2–4 days old, but 14–16-day-old males were less competitive than normal males of the same age (White, 1966). Grover & Pillai (1970) using larval treatment in their studies on the same mosquito found that hempa-sterilized males were fully as competitive as normal males for mating with normal females. Similar results were obtained by LaBrecque etal. (1962, 1966) with apholate- and hempa-sterilized males of the housefly. Crystal (1965, 1969), working with *C. hominivorax*, observed that males sterilized by *N, N'*-tetramethylenebis (1-aziridinecarboxamide) surpassed normal males in mating competitiveness by a factor of at least 4.

Ascher (1964) coined the term " hypercompetitiveness " to describe the phenomenon of inducing higher actual sterility than theoretically expected in mating competitiveness tests. Various explanations have been advanced for this observed phenomenon. Whiting & von Borstel (1954), on the basis of their findings with *Bracon hebetor* Say treated with mechlorethamine, suggested that the treated sperms were " stimulated " to fertilize more eggs than the normal sperms. However, LaBrecque, Meifert & Smith (1962) thought that either apholate-sterilized male houseflies may have super-normal sexual vigour or second matings with sterilized males may tend to nullify the effect of a first mating with fertile males. The former possibility was supported by Crystal's (1969) studies with screw-worm flies. Yet another suggestion, advanced by Ascher & Avdat (1967), is that some of the matings with male houseflies sterilized by *m*-xylohydroquinone might have been with oligospermic or aspermic individuals. But exactly how a chemosterilant enhances the mating ability of males is yet unknown. Probably chemosterilants trigger some neural or humoral mechanisms which promote sexual attraction (Crystal, 1969).

Reports are also available indicating that chemosterilization has reduced the mating ability of males, as shown with apholate- and metepa-sterilized males of *Ae. aegypti* and metepa-sterilized males of *An. quadrimaculatus* (Dame, Woodward & Ford, 1964; Dame & Schmidt, 1964). Similarly, males of the screw-worm fly sterilized by tretamine and uredepa failed to compete equally with normal males and could not inseminate as many females as normal males could (Crystal, 1964a, 1964b). Chang (1965) observed that males of the housefly sterilized by tepa were less competitive than normal males. Das (1967) considered that the results obtained by Dame & Schmidt (1964) with *Ae. aegypti* might be due to some specific effect of metepa. It is interesting to note that chemosterilization by tarsal contact impaired the sexual competitiveness of males of *Ae. aegypti*, *An. quadrimaculatus* and *M. domestica* when they were exposed to surfaces treated with metepa; however, when metepa was administered orally the treated males were as competitive as normal ones (Dame & Schmidt, 1964). Similarly,

larval treatment with apholate resulted in loss of vigour in males of *Ae. aegypti*, whereas apholate fed to adults of this mosquito did not affect the mating ability of males (Dame, Woodward & Ford, 1964; Weidhaas & Schmidt, 1963). In *C. p. fatigans* larval treatment with apholate in the present studies did not affect the mating ability of males as it did for *Ae. aegypti*. It is probable that the mating ability of a chemosterilized male is an independent factor governed by interaction of the chemical and the insect. This may further be influenced by the dose of the chemosterilant applied, the stage and duration of treatment, the mode of application and nutrition, as LaChance, North & Klassen (1968) have suggested.

Although the insemination rate for males of *C. p. fatigans* treated with apholate or tepa was similar to that for normal males in the first 48 hours, further (unpublished) studies of ours have indicated that such treated males depleted their sperms earlier than normal males so that their efficiency in insemination declined gradually. Similar early sperm depletion has been reported in *Ae. aegypti* treated with apholate by Sharma & Rai (1967) and with hempa by Grover & Pillai (1970). These authors have suggested that such aspermic males may not be disadvantageous if they can transfer a certain amount of seminal fluid into the virgin wild females. This fluid will make subsequent insemination by wild males into such females impossible; Craig (1967) has shown in *Ae. aegypti* that the secretion of the male accessory gland makes the female monogamous and prevents further insemination.

The analysis of the percentage hatch classes of egg-rafts clearly indicates that in *C. p. fatigans* the eggs are fertilized by the sperms transmitted in the first mating, though the female may mate several times. This fact is further proved by the mating sequence experiment, where the first mating always decided the fate of the majority of eggs. However, 5% of females in the last two crosses showed a percentage hatch which appears to be due to the second mating. It is probable that these females had some behavioural difference from the rest in not being ready to mate in the first 48 hours. Thus what appears to be the second mating for these females might in reality have been the first. Identical results were obtained with *Ae. aegypti* by George (1967) using irradiated males and by Grover & Pillai (1970) using hempa-sterilized males. Knipling (1955) and von Borstel (1960) assumed that in polygamous insects spermatozoa from different matings have an equal opportunity to fertilize ova and on this assumption they suggested that the "sterile-male technique" could be effective even against polygamous insects. Males of *An. pharoensis* Theobald, sterilized by ^{60}Co were able to nullify, to some extent, the previous insemination by normal males; but second insemination by normal males could not nullify insemination by irradiated males (Abdel-Malek, Tantawy & Wakid, 1967). On the other hand, Bryan (1968), using sterilized males of *Anopheles gambiae* species B, found that the first mating is the more important for fertilizing eggs. Similar studies on *An. quadrimaculatus* also did not produce any evidence that multiple matings occur extensively in the field (Dame, Woodward, Ford & Weidhaas, 1964). With *Ae. aegypti*, Dame, Woodward & Ford (1964), using apholate treatment at the larval stage, found that in a second mating with normal males sperms could to some extent nullify the sterilizing impact of a first mating, while in a reverse mating, treated sperms could not show any impact on a first mating with normal males. However, studies of other workers on this species have shown that normally this does not happen (Weidhaas & Schmidt, 1963; George, 1967; Grover & Pillai, 1970). Thus our present findings with *C. p. fatigans* do not support Knipling's suggestion. Studies by Kitzmiller & Laven (1958) using genetic markers have revealed that the eggs of this mosquito are fertilized by the sperms of only one male.

Field experiments to control mosquitos by the use of chemosterilants hold promise and are being attempted on *C. p. fatigans*. For any such field experiment to control *C. p. fatigans*, it will be essential to have basic biological information regarding the mating acceptability of normal or laboratory sterilized males by wild females. From the present studies with a wild population, it is quite certain that sterilized laboratory males of *C. p. fatigans* are not only compatible with wild females but could even induce the same level of sterility in wild females as they induce in laboratory females. Furthermore, the finding that laboratory males sterilized by apholate were more competitive in mating with wild females than were wild males is very important for the prospects of this chemosterilant in the field. However, it is yet to be ascertained whether sterilized males that are competitive with wild males for wild females when allowed to mate in mosquito cages will behave the same way in field conditions. Studies on the release of *An. quadrimaculatus* and *Ae. aegypti* males sterilized by gamma-radiation into natural populations

revealed no reduction of the natural population (Weidhaas, Schmidt & Seabrook, 1962; Morlan, McCray & Kilpatrick, 1962). These authors stressed the need for additional investigations on the biology of these mosquitos, especially on the dispersion of males in the field and on their mating ability with wild populations. Dame, Woodward, Ford & Weidhaas (1964) conducted a series of experiments to study the mating behaviour of sterile male *An. quadrimaculatus* in the field and found that laboratory males sterilized by apholate or tepa or by radiation were sexually vigorous and competitive with wild males for laboratory females and also sexually compatible with wild females. However, their lack of mobility or ability to seek out and inseminate females and perhaps certain other behavioural factors brought on by colonization probably hampered their success to induce sterility in wild females.

Recently, two field experiments to control *C. p. fatigans* have been made by Patterson and coworkers [1] on the island of Sea Horse Key off the coast of Florida by the release of males sterilized with tepa or thiotepa. In the first experiment, conducted in 1968, it was found that tepa-sterilized males were fully competitive with wild males even when the sterilized males were released 120 m away from the main breeding-sites and that 85% of the egg-rafts collected 8 weeks after release were sterile. In the second trial, in 1969, the island population of *C. p. fatigans* was completely eliminated within 12 weeks of the release of thiotepa-sterilized males. Field studies on the dispersal of ^{32}P-tagged *C. p. fatigans* in Burma have been recently conducted by Lindquist et al. (1967). Similar data on the dispersion of sterile males would be useful in assessing their ability to inseminate females in the field. Since females of *C. p. fatigans* may mate several times but their eggs are fertilized only by the first mating, it might be an advantage for sterile-male releases to be made at a time when the majority of the female population is virgin. Such releases should be made close to the breeding-places, so that treated males get the same opportunity as wild males to fertilize virgin females.

[1] Patterson, R. S., Weidhaas, D. E., Ford, H. R. & Lofgren, C. S. (1970) *Suppression and elimination of an island population of* Culex pipiens fatigans *with sterile males* (unpublished working document WHO/VBC/70.180). A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

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RÉSUMÉ

APTITUDE À L'ACCOUPLEMENT CHEZ *CULEX PIPIENS FATIGANS* WIEDEMANN MÂLE STÉRILISÉ PAR L'APHOLATE ET LE TEPA

Des recherches de laboratoire ont montré que certains produits chimiques comme l'apholate, appliqué au stade larvaire, et le tepa, appliqué au stade nymphal, provoquent un taux élevé de stérilité chez *Culex pipiens fatigans* mâle. L'utilisation sur le terrain des mâles ainsi traités ne peut cependant être envisagée que s'ils sont capables de soutenir la concurrence sexuelle des mâles sauvages normaux et, chez les espèces polygames, de fournir un sperme actif pouvant rivaliser avec le sperme des insectes non traités.

On a mené une série d'expériences de laboratoire à l'aide de *C. p. fatigans* stérilisés par exposition, au stade larvaire, à 10 parties par million d'apholate ou, au stade nymphal, à 3580 parties par million de tepa. En utilisant des insectes d'élevage, on a relevé des taux d'insémination de femelles normales très semblables pour les mâles stériles et pour les mâles normaux. Soumis à la concurrence sexuelle, les *Culex* mâles stérilisés par l'apholate ont montré des aptitudes supérieures à celles des mâles normaux. Lorsque des femelles vierges ont subi des accouplements successifs — d'abord avec des mâles stériles puis avec des mâles normaux, ou vice versa —

seul le premier accouplement a généralement été fécondant. Les taux d'insémination de femelles sauvages par des mâles d'élevage stérilisés ou par des mâles sauvages normaux ont été quasi identiques. Mis en même temps que des mâles sauvages normaux en présence de femelles sauvages, les mâles d'élevage stérilisés par l'apholate ont fait preuve d'aptitudes sexuelles équivalentes.

Les incidences de ces données expérimentales en ce qui regarde les applications pratiques sont brièvement évoquées.

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